Preliminary

Molecular Cloning and Sequence Analysis for Δ-Pyrroline-5-Carboxylate Synthetases from Mangrove Plant

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Introduction

Mangrave plants form forests in tropical and subtropical seashore. Growing in the intertidal region, mangrove plants are frequently exposed to high concentration of salt. Many plants accumulate sugar alcohols or low molecular weight compounds in response to salt stress. These compounds have been referred to compatible solutes. In some mangrove species, one of the compatible solute, proline is synthesized and accumulated in cells under salt stress[1,2). Proline is synthesized from glutamic acid via two successive reductions[3,4). The first step is catalyzed by a Δ-pyrroline-5-carboxylate synthetase (P5CS) that appeared to be induced by salt stress[5). Proline accumulation increases under the condition of high salt concentration in transgenic plant introduced P5CS gene[6). There have been many physiological and morphological reports on the mechanisms of salt tolerance of mangrove. However there are few reports on the mechanisms of salt tolerance at molecular level in mangrove plants.

As a step towards understanding the mechanism of salt tolerance at the molecular level in mangrove plants, P5CS gene fragment was isolated from mangrove *Bruguiera sexangula* genomes by the polymerase chain reaction (PCR). In this paper, we report here the isolation and sequence analysis of P5CS gene fragment from the mangrove plant *B. sexangula.*

Materials and Method

Mangrove plants *(Bruguiera sexangula)* were grown for about four years in soil under 16h light conditions at 28°C.

Gemomic DNA was isolated from young leaves based on a CTAB (Cetyltrimethyl

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1    GGAAGAGGAGGCATGACTGCCAAAGTCAAAGCTGCTGTTAGTGCTGCTAATGCTGGCACC
     G  R  G  G  M  T  A  K  V  K  A  A  V  S  A  A  N  A  G  T
61   CCTGTTGTTATAACAAGgtatcctttgtgctatattctcttacatggttctgaatatggc
     P  V  V  I  T  S
121  atccaaaattagtgaatacttatgttcccttctatgttggagattgtcggaagtttagtg
181  gcatgcctgtttggtgccattcttgtcttgagagcttttagtttctaatgtggcaattta
241  tttttttcttcttgtcgtgctgtgtgatgctgcctactgaatgaatggggcaaagagat
301  atctgaccctccatttttttatatccagtgtgaacattatcttatacgtcagcctcctg
361  tttatctgcttgcatatttgttgcgggtctggctctgttgtttctctctctcttcttttt
421  ccatttatttgcttacatctttgatgtctgtttggttctattgtttcttaagTGGGAATG
                                                          G  N  A
481  CTCCAGAAAACATCATCAAAGTCCTTCAAGGAGAGCGTGTTGGCACACTCTTTCGCAAAG
      P  E  N  I  I  K  V  L  Q  G  E  R  V  G  T  L  F  R  K  D
541  ATGCACATTTGTGGGCCTCAGTTAAAGAAGTTGGTCCACGTGAGATGGCACTTGCAGCAA
      A  H  L  W  A  S  V  K  E  V  G  P  R  E  M  A  L  A  A  R
601  GGGAAAGTTCAAGACAGCTCCAGgtatcatcactttggagcatggctgcaagattttacg
      E  S  S  R  Q  L  Q
661  ttcttatgctgttgcanaacaattttgttccctctgcaaattgaaatacactttgtctta
721  caatggtttaacttattcactacggcataataatgtatagtatatgtctccctagGCACT
                                                             A  L
781  GTCATCTCAAGATAGGAAAAAAATATTGCTGGGTATTGCTGGGGCCCTGGAGGCCAATGA
     S  S  Q  D  R  K  K  I  L  L  G  I  A  G  A  L  E  A  N  E
841  AAATTTGATCAAAATTGAAAATGAAGCTGACGTTGCTGCTGCGCAGGAGGTTGGATTGGA
     N  L  I  K  I  E  N  E  A  D  V  A  A  A  Q  E  V  G  L  E
901  AAAATCATTAATTTCTCGATTGGCTCTAAAGCCTGGGAAGgcaagattttgttggtttat
     K  S  L  I  S  R  L  A  L  K  P  G  K
961  tttaaaattttcactttatatggttttgatttttctatatatttcttggtgtttgttttg
1021 aatcttcaggtttgtaactagcttattcattgtctttttttttttttttgtttatttcna
1081 atataacattggctggttctatgccaacctctctctctttctcttttattctttctcttc
1141 tctaaatacacttttttttaagccttttcatctggttaactttcaattaatattaacagA
                                                                I
1200 TTACAAATCTAGCAAATTCAATACGAGTGCTTGCTGACATGGAAAATCCAATTGGCCATA
      T  N  L  A  N  S  I  R  V  L  A  D  M  E  D  P  I  G  H  I
1261 TTTTGAAAAGGACTGAGgtcagaaaatgtttatatcccccatgcatttgctcatggtttt
      L  K  K  T  E
1321 gaagaattttaatctgcttctatgtgttttgacacaataagggtaaagcaagttttgaca
1381 ataatctgcaattggtagtaagagatgcttagatgttgtagagtgagactgtaaccaaat
1441 ttttggacattttgaaacctcataaaatgactgatttcctcttgtccagatcacttctt
1501 tgtgatgtttctgaatactatcatgcattcagGTTGCAGATGGACTCCTCTTAGAGAAGA
                                     V  A  D  G  L  L  L  E  K  T
1561 CATGCTCTCCTTTAGGTGTCCTCCTGATAGTTTTTGAGGCTCGACCAGAGGCACTAGTAC
      C  S  P  L  G  V  L  L  I  V  F  E  A  R  P  E  A  L  V  Q
1621 AGgtacctgaagaaatgtgtctgaagctcatctgtactaatctcttatttgttgatcttt
1681 tagtgaaggttggataaagttaggatctagcattagcaacttcagtggctgctcacttca
1741 tacaaacatattcatgtggttattattccattactgtaggtttgcagaggcttaaagcct
1801 gactggcaatttctttctctctagtgttctatttcattgtgtttttttaacccttttttttt
1861 tttttttggttgattgaatagATAGCTTCGTTAGCAATCCGAAGTGGGAATGGGCTCCT
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                                       I   A   S   L   A   I   R   S   G   N   G   L   L
1921 CTTAAAAGGGGGAAAGGAGGCCATGCGGTCTAATGCAATCTTGCACAAGgcaagtttctt
      L   K   G   G   K   E   A   M   R   S   N   A   I   L   H   K
1981 taaattcatcttcattttttgtggagaatatcagtctactgataatgtatgcatgggctta
2041 tattgtcaaattatttctaaagttttgtcattcttgttgtatttatagGTTATCACTGAA
                                                     V   I   T   E
2101 GCCATTCCAGACACCATTGGGGCCGGACTTATTGGACTGGTGACATCGAGGGAGGAAATT
      A   I   P   D   T   I   G   A   G   L   I   G   L   V   T   S   R   E   E   I
2161 CCTGATCTGCTCAAGgtgagtaggtcttctagagactattgattacctgtacaatgtcca
      P   D   L   L   K
2221 ctccaaagaactcactctcctttttggcaccagCTTGATGATGTGATTGATCTAGTGAT
                                       L   D   D   V   I   D   L   V   I
2281 CCCAAGAGGCAGCAATAGACTCGTTACTCAAATTAAGGAATCAACTAAAATCCCTGTTTT
      P   R   G   S   N   R   L   V   T   Q   I   K   E   S   T   K   I   P   V   L
2341 GGGTCATGCTGgtgagatggattatatttaggatcctattggttgaaagtgatgtctgat
      G   H   A   D
2401 ctttgtagtaaagcttcattttgtgctgtacagATGGGATTTGTCATGTTTACATTGACA
                                       G   I   C   H   V   Y   I   D   K
2461 AGTCTGCTAACATGGAAATGGCAAGCCGTGTTGTTTTGGATGCAAAATTAGATTATCCAG
      S   A   N   M   E   M   A   S   R   V   V   L   D   A   K   L   D   Y   P   A
2521 CAGCCTGCAATGCGATG
      A   C   N   A   M
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Fig. 1. Nucleotide and deduced amino acid sequences of the P5CS gene amplified from *B. sexangula* genomic DNA by PCR.

ammonium bromide) method used CTAB solution as extraction buffer. Partial DNA fragment encoding P5CS was amplified by polymerase chain reactions (PCR). The primers for PCR were designed according to the sequence in 6 exon and 14 exon of P5CS gene conserved highly among *Arabidopsis thaliana*[7)], *Vigna aconitifolia*[8)], *Oryza sativa*[9)] and *Solanum lycoperscicum*[10)]. The PCR products were cloned into T-vector and sequenced by dideoxy chain termination method[11)].

Results and Disscussion

The cloned PCR products were sequenced completely on the both strands, which revealed that the cloned DNA was 2,537 bp in length, approximately 500 bp longer than P5CS DNA between two position corresponding to primer sequence for PCR from *A. thaliana* (Fig. 1). The partial DNA fragment was contained putative 9 exons, as expected from the primers designed from the sequence of full-length P5CS gene from *A. thaliana*[7)]. The sizes of exons were completely corresponded with those of the *A. thaliana*. The nucleotide sequence and deduced amino acid sequence of *B. sexangula* showed homologies (60–90%) with the P5CS cDNA of *A. thaliana*[7)], *V. aconitifolia*[8)], *O. sativa*[9)], *S. lycopersicum*[10)] and *A. deliciosa*[12)], *Medicago sative* (accession no. X98421). The amino acid sequence of 11 and 12 exons showed especially high similarity (80–90%) with those encoded by known genes for

NADPH-binding domain from *A. thaliana*[7], *V. aconitifolia*[8], *S. lycopersicum*[10] and *A. deliciosa*[12]. These results indicate that the conservation of P5CS gene is high among herb and woody plants, such mangrove plant growing in severe condition as seashore.

The introns of partial P5CS gene from *B. sexangula* was contained T-rich sequences characteristic of intron sequence and its sizes was found to be almost same or 100–200 bp longer than that of the *A. thaliana*.

In this study, we first succeeded in the isolation of partial DNA fragment of P5CS gene from mangrove plant by PCR method.

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